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Abstract: Tumour immunotherapy, and particularly immue checkpoint inhibitors, have resulted in considerable response rates in patients with metastatic cancer. However, most of these approaches are limited to immunogenic tumours. Based on its ability to stimulate cytotoxic T cells, interleukin-2 (IL-2) has been used to treat patients with metastatic melanoma and metastatic kidney cancer. Clinical efficacy achieved through high doses is countered by severe adverse effects on vascular endothelial cells and various organs, a short in vivo half-life, and the stimulation of regulatory T cells that counteract antitumour immune responses. Accumulating evidence suggests that IL-2 receptor beta; (CD122)-biased IL-2 formulations address the shortcomings of IL-2 cancer immunotherapy. This knowledge stems from studies using CD122-biased IL-2/anti-IL-2 antibody complexes (IL-2 complexes), which preferentially stimulate CD8+ T cells, while interaction with regulatory T cells and vascular endothelial cells is disfavoured by the anti-IL-2 antibody used. CD122-biased IL-2 complexes, when assessed in different mouse cancer models, cause stronger antitumour effects and significantly less adverse effects than high-dose IL-2. A recently developed and characterised anti-human IL-2 antibody, termed NARA1, forms human CD122-biased IL-2 complexes. Alternative strategies based on this concept, such as site-directed pegylation and mutation of IL-2, have also been pursued. Moreover, recent data have shown that a combination of CD122-biased IL-2 formulations with immune checkpoint inhibitors, antigen-specific immunotherapy and epigenetic modifying drugs results in synergistic anti-cancer effects in various tumour models. Thus, CD122-biased IL-2 approaches constitute a novel class of immunotherapy for metastatic cancer that has the potential to complement and increase the efficacy of other antitumour strategies.

Development of a novel class of interleukin-2 immunotherapies for metastatic cancer

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Summary

Tumour immunotherapy, and particularly immue checkpoint inhibitors, have resulted in considerable response rates in patients with metastatic cancer. However, most of these approaches are limited to immunogenic tumours. Based on its ability to stimulate cytotoxic T cells, interleukin-2 (IL-2) has been used to treat patients with metastatic melanoma and metastatic kidney cancer. Clinical efficacy achieved through high doses is countered by severe adverse effects on vascular endothelial cells and various organs, a short in vivo half-life, and the stimulation of regulatory T cells that counteract antitumour immune responses. Accumulating evidence suggests that IL-2 receptor β (CD122)-biased IL-2 formulations address the shortcomings of IL-2 cancer immunotherapy. This knowledge stems from studies using CD122-biased IL-2/anti-IL-2 antibody complexes (IL-2 complexes), which preferentially stimulate CD8^{+} T cells, while interaction with regulatory T cells and vascular endothelial cells is disfavoured by the anti-IL-2 antibody used. CD122-biased IL-2 complexes, when assessed in different mouse cancer models, cause stronger antitumour effects and significantly less adverse effects than high-dose IL-2. A recently developed and characterised anti-human IL-2 antibody, termed NARA1, forms human CD122-biased IL-2 complexes. Alternative strategies based on this concept, such as site-directed pegylation and mutation of IL-2, have also been pursued. Moreover, recent data have shown that a combination of CD122-biased IL-2 formulations with immune checkpoint inhibitors, antigen-specific immunotherapy and epigenetic modifying drugs results in synergistic anti-cancer effects in various tumour models. Thus, CD122-biased IL-2 approaches constitute a novel class of immunotherapy for metastatic cancer that has the potential to complement and increase the efficacy of other antitumour strategies.

Keywords: IL-2, cytokine, immunotherapy, tumour, cancer, melanoma, renal cell carcinoma

Introduction

Cancer is a major health problem of our time. With an estimated 8.8 million deaths globally in 2015, cancer is the second leading cause of death worldwide [1]. Moreover,

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the economic impact of cancer in 2010 was estimated at about 1.16 trillion US dollars, further emphasising the urgency and importance of fighting this disease. Whereas localised cancer can often be cured using surgery and radiation, metastatic disease, where cancer cells spread to distant sites of the body, is often incurable. The current treatment options for metastatic cancer include, in addition to the aforementioned approaches, chemotherapy, agents targeting cancer-associated pathways, angiogenesis inhibitors and tumour immunotherapy. In fact, the recent impact of immune checkpoint inhibitors on advanced cancer has firmly established the importance of tumour immunotherapy in the treatment of metastatic disease [2].

However, stimulation of the immune system to fight cancer is not a new concept. In the late 19th century, the New York surgeon William Coley injected cancer patients intratumourally with bacterial extracts, with the aim of eliciting an antitumour immune response [3]. Yet almost a century separates Coley's initial clinical trial from the discovery of tumour antigens and the use of the first molecularly defined immunotherapies, including interleukin-2 (IL-2), for metastatic disease [4, 5]. In 2010, the use of a monoclonal antibody (mAb) blocking the immune checkpoint cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) was reported to show a significant improvement in the overall survival of patients with metastatic melanoma [6], leading the field towards the research and development of immune checkpoint inhibitors. While the clinical use of immune checkpoint inhibitors and the science behind them are transforming tumour immunotherapy and immunology [2], IL-2-based immunotherapy does provide some advantages that justify renewed interest in this approach against advanced cancer.

IL-2 immunotherapy

IL-2 was discovered, purified and molecularly characterised between 1976 and 1983 [7–9]. A small cytokine of 15.5–16 kDa consisting of four α-helices, IL-2 is a member of the common cytokine receptor γ chain (γc) that also includes IL-4, IL-7, IL-9, IL-15 and IL-21 [10]. In the early 1990s, IL-2 was the first immunotherapy used and approved in metastatic melanoma and metastatic renal cell carcinoma. In these indications, high-dose IL-2 ther-

apy results in an overall response rate of 15-17% and in about 5-10% durable and complete disease remission [5]. A course of high-dose IL-2 therapy consists of two cycles of 600,000–720,000 international units/kg, given every 8 hours for up to 12-15 doses per cycle, and further courses of treatment are repeated every 2 months if tolerated by the patient [11]. Therapeutic response rates can increase to 30-70% when IL-2 is combined with adoptive T cell transfer together with nonmyeloablative conditioning or with cancer vaccines.

Despite these promising outcomes, IL-2-based approaches have not been widely adopted. The reasons for this include the short half-life of IL-2, which makes its administration to patients inefficient, its dose-dependent, toxic adverse effects on the vasculature and other organs, and its stimulation of regulatory T (Treg) cells that dampen antitumour immune responses [12, 13]. A previously held conviction paralleled the adverse effects of IL-2 with its antitumour activities. It was thought that IL-2 activated cytotoxic T cells and natural killer (NK) cells to attack cancerous cells but, simultaneously, the same cells released vaso-active substances, including tumour necrosis factor (TNF), that resulted in vascular (or capillary) leak syndrome (VLS) [14–19]. However, in 2010 we were able to demonstrate that IL-2-mediated toxicity and VLS was the result of a two-step process, whereby, firstly IL-2 binds to and damages endothelial cells, and in a second phase, IL-2-activated T and NK cells release pro-inflammatory cytokines including TNF [20]. The association of IL-2 with endothelial cells is facilitated by the expression of IL-2 receptor α (IL-2Rα, also called CD25) on endothelial cells, the levels of which further increase upon IL-2 immunotherapy in vivo, whereas the antitumour activity of IL-2 is independent of CD25 but requires cells expressing IL-2Rβ (also termed CD122) and γ_c [20]. These findings opened up the possibility of using modified IL-2 approaches, such as CD122-biased IL-2/anti-IL-2 monoclonal antibody complexes (IL-2 complexes; see below) [13, 21–23] and IL-2 muteins [24]. Our 2010 Krieg et al. report also motivated Charych and colleagues [25] to PEGylate IL-2 with the aim of biasing IL-2 to preferentially bind to CD122 [26]. These approaches are based on the differential expression of IL-2R subunits, as discussed in the next chapter.

IL-2 approaches biased to different IL-2 receptors

IL-2Rs include dimeric IL-2Rs consisting of CD122 and γ_c, or trimeric IL-2Rs made of CD122, γ_c and CD25 [12, 13]. The CD122–γ_c heterodimer mediates signal transduction. Conversely, CD25 by itself can serve as a non-signalling, low-affinity IL-2R, with a dissociation constant (K_d) of about 10^{-8} M. Upon association with CD25, thus forming the trimeric IL-2R, the binding property of the dimeric CD122–γ_c IL-2R increases from intermediate (K_d about 10^{-9} M) to high affinity (K_d about 10^{-10} M to 10^{-11} M) [13]. Thus, the principal function of CD25 appears to be to increase the affinity of the IL-2R. However, CD25 can also exert other functions. CD25 is prominently expressed on Treg cells, where the excess of CD25 could serve as a sink, thereby taking IL-2 away from effector T cells [27, 28]. Moreover, as mentioned above, endothelial cells express CD25, maybe to sense IL-2 signals during in-

flammation, thereby locally increasing the leakiness of the vasculature to allow influx of leukocytes and to concentrate IL-2 molecules on their surface for transiting T cells [20, 28].

In the context of cancer immunotherapy, IL-2 formulations that allow IL-2 binding to the dimeric CD122–γ_c IL-2R but disfavour the association of IL-2 with CD25 (thus termed CD122-biased) could be advantageous. Strategies to obtain CD122-biased IL-2 formulations that have been pursued include, to name but a few, (i) CD122-directed IL-2 complexes, in which the anti-IL-2 mAb covers the CD25-binding site (fig. 1A–C), (ii) IL-2 muteins with a mutation at the CD25-binding site, (iii) IL-2 carrying polyethylene glycol (PEG) groups at the CD25-binding site and (iv) a fusion protein of IL-2 and CD25. PEGylation requires exclusive targeting of the IL-2 residues involved in CD25 interaction, which so far has not been achieved. Thus, PEGylation of accessible lysine (K) residues of human IL-2, as in NKTR-214 [26], will also target epitopes that are involved in CD122–γ_c binding [13]. A CD25–IL-2 fusion protein consists of two functional parts that interact with each other with low affinity (10^{-8} M), unlike the CD25 mimobody NARA1 (see below). IL-2 muteins have the advantage of consisting of only one molecule, but they are considerably immunogenic and have a short half-life [13]. Conversely, CD122-directed IL-2 complexes are made of natural (i.e. non-immunogenic) IL-2 bound by a high-affinity mAb. While natural IL-2 has a short in vivo half-life, measured in minutes, due to rapid renal clearance, CD122-directed IL-2 complexes have an in vivo half-life of days [21, 23]. The increased in vivo half-life is the result of the association of the small (15.5-16 kDa) IL-2 with an antibody of 150 kDa [23], and this principle also applies to other cytokines, including IL-1β, IL-3, IL-4, IL-6, IL-7, IL-15, interferon-γ, and granulocyte colony-stimulating factor [22, 30–38]. IL-2 complexes, in comparison to complexes made of most other cytokines, are particular because, depending on the anti-IL-2 mAb used, they preferentially stimulate either CD122^{high} cells expressing CD122–γ_c dimeric IL-2Rs or CD25^{high} cells carrying trimeric IL-2Rs. This principle, first described in 2006 [22], states that CD122-biased IL-2 complexes (e.g. mouse IL-2/S4B6 complexes or human IL-2/NARA1 complexes) preferentially stimulate CD122^{high} cells, including antigen-experienced (memory) CD8^{+} T cells and NK cells. Conversely, CD25-biased IL-2 complexes (e.g. mouse IL-2/JES6-1 complexes or human IL-2/5344 complexes) selectively activate CD25^{high} cells such as thymus-derived CD25^{high} Treg cells [12, 13, 19]. Mechanistically, mAbs that form CD122-biased IL-2 complexes bind to and cover IL-2 epitopes that usually interact with CD25, whereas mAbs that form CD25-biased IL-2 complexes associate with and temporally obstruct IL-2 epitopes that interact with CD122 and γ_c [13].

CD122-directed IL-2 complexes have been shown in several preclinical cancer models to exert superior antitumour responses compared to IL-2, either as a monotherapy or in combination with other immune stimulating agents, cancer vaccines and epigenetic modifying agents [20, 21, 24, 39–42]. Cancer types where CD122-biased IL-2 formulations have shown efficacy include melanoma (B16F10 and *Tyr::N-Ras[Q61K] Ink4a^{−/−}* mice), lung carcinoma (Lewis

lung carcinoma), colon carcinoma (MC38), prostate carcinoma (TRAMP-C1), sarcoma (MCA-205) and leukaemia (BCL1) [20, 21, 24, 39–43] (fig. 1D–F). As for epigenetic modification, a combination of IL-2/NARA1 complexes with an inhibitor of enhancer of zeste homologue 2 (EZH2) was particularly advantageous. Thus, systemic or tumour-intrinsic EZH2 inhibition in different mouse models of melanoma was able to reverse the features of adaptive resistance to tumour immunotherapy such as silencing of immunogenicity and antigen presentation, as well as upregulation of programmed death ligand 1 (PD-L1, also known as CD274) on melanoma cells [42]. Recently, a monoclonal anti-human IL-2 antibody, termed NARA1, has been generated and characterised. It forms CD122-directed human IL-2 complexes and is suitable for clinical development [21]. Because it mimics CD25's binding to the CD25 epitope of human IL-2, NARA1 was also dubbed a CD25 mimobody. However, unlike CD25, NARA1 binds with high affinity (10^{-9} M) to IL-2. Similar to their mouse analogues, IL-2/NARA1 complexes preferentially stimulate CD8$^+$ T cells and NK cells while sparing Treg cells and endothelial cells, and show strong efficacy in several mouse tumour models [21] (fig. 1D–F).

CD122-biased IL-2 therapies currently in development are listed in table 1.

Concluding remarks

As CD122-biased IL-2 formulations are being tested in patients, the first clinical data are becoming available, including potential advantages and disadvantages [13, 44, 45]. Due to its different but proven mode of action, IL-2 immunotherapy could complement many anti-cancer approaches, including immune checkpoint inhibitors, adoptive cell transfer regimens and vaccination, as well as strategies that are not considered typical immunotherapies (such as epigenetic modifying agents), to name a few. In adoptive cell transfer approaches, CD122-biased IL-2 complexes could help to rescue T cells that have been
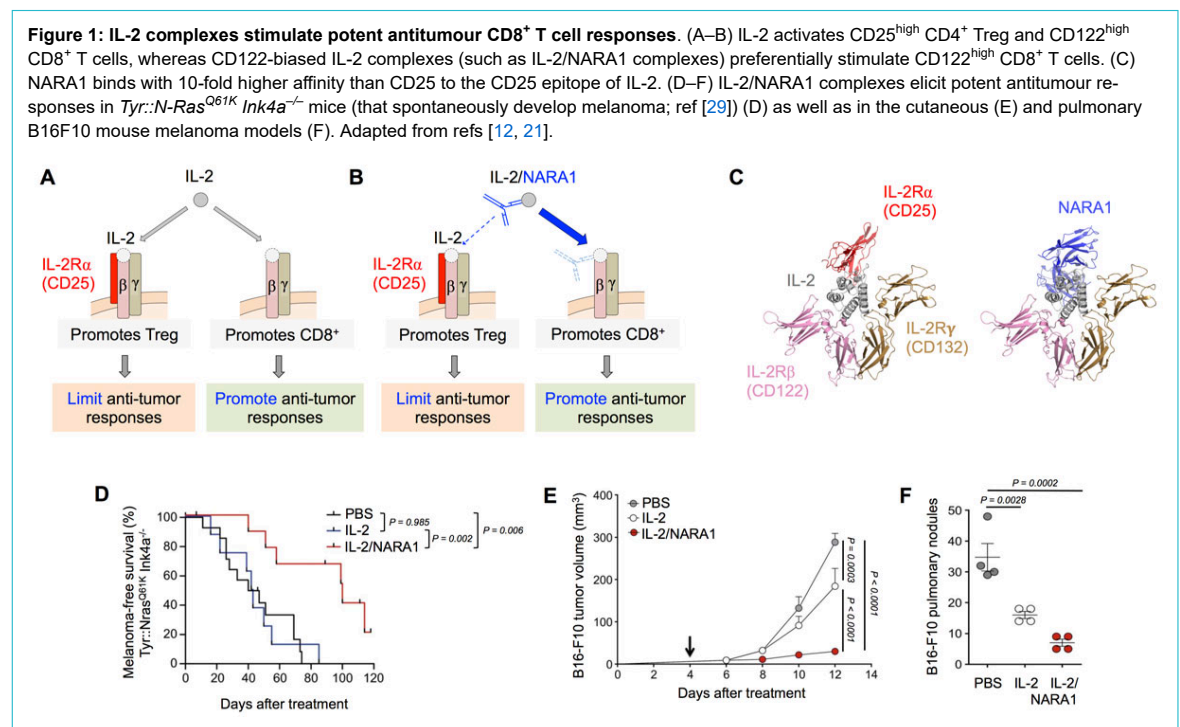

Figure 1: IL-2 complexes stimulate potent antitumour CD8$^+$ T cell responses. (A–B) IL-2 activates CD25^{high} CD4$^+$ Treg and CD122^{high} CD8$^+$ T cells, whereas CD122-biased IL-2 complexes (such as IL-2/NARA1 complexes) preferentially stimulate CD122^{high} CD8$^+$ T cells. (C) NARA1 binds with 10-fold higher affinity than CD25 to the CD25 epitope of IL-2. (D–F) IL-2/NARA1 complexes elicit potent antitumour responses in *Tyr::N-Ras^{Q61K} Ink4a$^{-/-}$* mice (that spontaneously develop melanoma; ref [29]) (D) as well as in the cutaneous (E) and pulmonary B16F10 mouse melanoma models (F). Adapted from refs [12, 21].

Table 1: CD122-biased IL-2 therapies in development.
Human IL-2 (hIL-2)-based formulations that have been reported to show an IL-2 receptor β (CD122) bias, i.e. preference of CD122 over IL-2 receptor α (CD25) binding, are listed according to their state of development.

Product (Company)	CD122 bias	Description	Status	Ref.
NKTR-214 (Nektar)	+	hIL-2 PEGylated on lysine (K) residues, resulting in up to 11 PEGs per hIL-2 (6 on average), of which 6 are in or adjacent to the CD25 epitope (thus resulting in CD122 bias), and 3-4 are in or adjacent to the CD122 epitope (thus favouring CD25 bias)	6 Phase I to III studies	CT, [26]
RG7461 (RO6874281) (Roche)	++	hIL-2 carrying the mutations F42A, Y45A and L72G, located in the CD25-binding epitope of hIL-2 and fused to a mAb targeting fibroblast activation protein-alpha (FAP)	4 Phase I to II studies	CT, [44]
ALKS 4230 (Alkermes)	++	hIL-2–CD25 fusion protein consisting of complementary mutated hIL-2 and CD25	Phase I recruiting	CT
ANV329 & ANV629 = humanised NARA1 (Anaveon)	++	Humanised NARA1 mAb binding with high affinity and affinity-matured to the CD25 epitope of hIL-2	Pre-clinical	Anaveon, [21]

CD25 = IL-2 receptor α; CD122 = IL-2 receptor β; CT = clinicaltrials.gov (accessed on Sept 4, 2018); hIL-2 = human IL-2; mAb = monoclonal antibody; PEGylated, harbouring polyethylene glycol residues.

strongly activated *in vitro* and to keep these cells alive, as suggested by a number of preclinical studies [10, 39–41, 46]. Notably, IL-2 immunotherapy might even contribute to turning a "cold" (i.e. poorly immunogenic) tumour into a "hot" (i.e. immunogenic) one, e.g. by the upregulation of PD-L1 on cancer cells [45]. However, to date, we do not know how these different processes will best benefit from CD122-biased IL-2 complexes, or whether IL-2 signals will be required in the tumour and the tumour microenvironment, and/or in secondary lymphoid organs. Future preclinical and clinical studies will shed light on these open questions on the biology and use of IL-2 and biased IL-2 formulations.

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Potential competing interests

The authors declare competing financial interests: O.B. and N.A.R. are inventors on patents on NARA1, and O.B. is a founder and shareholder of Anaveon AG.

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